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Description	36
Claim(s)	2
Abstract	
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Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature J.Miller+Co Date

J. Miller & Co. 15.3.96

12. Name and daytime telephone number of person to contact in the United Kingdom

C. M. Sturt - 0171 242 5974

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference
   GBP11629B

2. Patent application number
   (The Patent Office will fill in this part)
   9605440.8

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*
   SCOTIA HOLDINGS PLC
   EFAMOL HOUSE
   WOODBRIDGE MEADOWS
   GUILDFORD
   SURREY GU1 1BA

   Patents ADP number *(if you know it)*

   If the applicant is a corporate body, give the country/state of its incorporation
   UK 02201400

4. Title of the invention
   PRESENTATION OF BIOACTIVES

5. Name of your agent *(if you have one)*
   J. MILLER & CO.

   "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*
   34 BEDFORD ROW
   HOLBORN
   LONDON WC1R 4JH

   Patents ADP number *(if you know it)* 1109002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
   a) any applicant named in part 3 is not an inventor, or
   b) there is an inventor who is not named as an applicant, or
   c) any named applicant is a corporate body.
   See note (d))
   Yes

Published Material

Concepts such as are discussed above have received no great attention in the publi: patent and general literature but there is material on certain specific natural diol derivatives and on nutritional and pharmaceutical uses of certain specific diol esters. A source paper in the general literature is Bergelson et al (Biochim., Biophys. Acta 116 (1966) 511-520) describing inter alia long chain di-esters of 1,3-propane diol. Little is said of the acid moieties but dioleates are identified. In the patent literature edible fat mimetics are for example proposed by Nabisco in EPA 0 405 873 and EPA 0 405 874 and include linolenic acid (this term indicating the "alpha" isomer when not qualified otherwise) and arachidonic acid esters of, apparently, 1,4-butane diol. Unilever's U.K. specification 2 161 477 (equivalent to EPA 0 161 114) concerns the growth and economic yield of plants, using inter alia 1,3-propane diol esters of linoleic acid and linolenic acid (again no doubt the alpha isomer). Anti-ulcer drugs of 2,3-butanediol esters are described in SS Pharmaceutical Co's EPA 0 056 189. Sundry pharmaceutical actions of propane-1,3-diol esters of short chain fatty acids are disclosed in Sanofi EPA 0 018 342. More distantly perhaps, Terumo K.K. in EPA 0 222 155 link 5-fluoro uracil to alpha linolenic acid, dihomo gamma linolenic acid, or eicosapentaenoic acid through a group -CH(R)-O- where R = methyl etc, as inter alia anti-cancer agents.

Lipid Barriers

Many drugs act at the cell membrane surface by combining with cell surface receptors, or alternatively are taken into cells by specific transport systems. However, there are many drugs which, while they act within cells by modifying one of many different functions such as nucleic acid functions, the actions of intracellular enzymes, or the behaviour of systems like the lysosomes or the microtubules, are not able to penetrate cells effectively. There may be no receptors and transport systems with which they can link, or these systems may transport the drug into the cell at a less then optimum rate. Equally drugs may penetrate intracellular membranes such as mitochondrial and nuclear membranes at less than optimum rates.

PRESENTATION OF BIOACTIVES

Field

The specification relates to the presentation of bioactives, in which term we include a drug, essential nutrient or any other compound to be administered to the human or animal body in therapy or maintenance of health.

In particular, the specification relates to the presentation of such bioactives in a form in which they are lipophilic so that they can pass lipid barriers in the body readily, or the presentation of two bioactives in the same molecule (where at least one of the bioactives is a fatty acid or fatty alcohol), or the presentation of such bioactives in a form which serves both aims and/or the aims of ready synthesis of a compound without a chiral centre. From a drug regulatory viewpoint it is a great advantage to have two bioactives presented as a single molecule rather than as two separate entities. There may also be advantages in presenting known bioactives in novel ways. Those advantages include increased lipophilicity, the additive effects of two bioactives which are not normally presented together, and the sometimes synergistic effects of such bioactives.

The invention concerns the linking of bioactives through certain link molecules, considered in detail later herein, and the synthesis of a range of compounds some of which are entirely novel in themselves, while others are novel in the sense of their usefulness in therapy and/or the maintenance of health. Discussion is however, also given of compounds using other link molecules, and of directly linked bioactives, disclosed for example in EPA 0 393 920 concerning fatty acids and antivirals, and in co-pending EPA 95301315.8 (published as EPA-0675103) concerning fatty acids and non-steroidal anti-inflammatory drugs.

2. Blood-brain barrier: all drugs acting on the central nervous systems will have their transport improved by this technique. This includes all drugs used in psychiatry, all drugs used in cerebral infections with any organism or cerebral cancer and all other drugs acting on nerve cells such as anti-epileptic drugs and others.

3. Skin: as with the blood-brain barrier, all drugs that may be required to penetrate the skin to achieve a systemic effect will benefit from their conversion to a fatty acid derivatives.

For example, the approach discussed is applicable to amino acids. Of particular interest are those which seem to play roles in the regulation of cell function as well as acting as components of proteins. Examples include tryptophan (a precursor of 5-hydroxytryptamine [5-HT], a key regular of nerve and muscle function), phenylalanine (a precursor of catecholamines) and arginine (a regulator of the synthesis of nitric oxide which also plays important roles in controlling cellular activities).

Properties Conferred Generally

Generally the compounds have many advantages in addition to their lipophilicity. Two moieties of a given fatty acid may be delivered in a form which is readily incorporated into the body as an oral, parenteral or topical formation, which is very well tolerated with none of the side effects associated, for example, with free fatty acids, which is not too stable to be properly utilised, which has no chiral centre, and which is much more readily synthesised than the corresponding triglyceride with three moieties of the same fatty acid attached. Whereas triglycerides are well tolerated and can be utilised, they are much less desirable than the proposed compounds because they are much more difficult to synthesise and have a chiral centre with multiple potential isomers.

There are other barriers to drug movements which are recognised as important. One of particular significance is the blood-brain barrier, which has many of the characteristics of the cell membrane. There are many drugs which have difficulty in reaching adequate concentrations in the brain because of this barrier. Another is the skin: until a few years ago drugs were applied to the skin only if their purpose was to act on the skin. However, it has been recognised that the skin can be an appropriate route for getting drugs with systemic actions into the body, and as a result more and more compounds are being administered by variations of patch technology.

All three types of barriers, the cell membrane and intracellular membranes, the blood-brain barrier and the skin have an important feature in common, they are substantially composed of lipids. What this means is that they are impermeable to primarily water-soluble drugs unless these drugs can be carried across the membrane by a receptor or transport system. In contrast, lipophilic substances are able to cross the barriers more readily without the need for any specific receptor or transport system.

Classes of Bioactives Requiring Passage Through Lipid Barriers

Drugs whose pharmacokinetic behaviour may be improved, listed by route of entry, are as follows:

1. Cell entry: drugs particularly likely to benefit are those that act primarily intracellularly. These include:

   a. All anti-inflammatory drugs, whether steroid or non-steroid
   b. All cytotoxic drugs used in the management of cancer;
   c. All antiviral drugs;
   d. All other drugs that have to enter cells in order to achieve optimum effects, in particular drugs which act on DNA or RNA, or on enzymes located intracellularly, or in second messenger systems, or on microtubules, mitochondria, lysosomes, or any other intracellular organelle.
   e. Steroid hormones and other hormones that act intracellularly, such as oestrogens, progestins, androgenic hormones and dehydroepiandrosterone.

indeed any fatty acid which has two or more cis or trans carbon-carbon double bonds, and use may be in the form of the fatty acid or the corresponding fatty alcohol. References to fatty acids are accordingly to be read herein as to both forms, except where the chemistry of one or the other specifically is under discussion. The desirable properties of GLA and DGLA however, make them especially valuable for the purpose.

FIGURE 1

n-6 EFA's		**n-3 EFA's**
18:2n-6 (Linoleic acid, LA)		18:3n-3 (α-Linolenic acid, ALA)
↓	δ-6-desaturase	↓
18:3n-6 (γ-Linolenic acid, GLA)		18:4n-3 (Stearidonic acid)
↓	elongation	↓
20:3n-6 (Dihomo-γ-linolenic acid, DGLA)		20:4n-3
↓	δ-5-desaturase	↓
20:4n-6 (Arachidonic acid, AA)		20:5n-6 (Eicosapentaenoic acid, EPA)
↓	elongation	↓
22:4n-6 (Adrenic acid)		22:5n-3
↓	δ-4-desaturase	↓
22:5n-6		22:6n-3 (Docosahexaenoic acid, DHA)

For example, in their own right GLA and DGLA have been shown to have anti-inflammatory effects, to lower blood pressure, to inhibit platelet aggregation, to lower cholesterol levels, to inhibit cancer cell growth, to reduce dyskinetic movements, to relieve breast pain, to improve calcium absorption and enhance its deposition in bone, to reduce the

When two different fatty acids are delivered, the advantages are as before plus the ability to administer simultaneously two materials with different biological actions in a single molecule. This avoids the regulatory problems which ensue when two materials are administered as separate compounds, as well as the issues which arise where there is the possibility of chiral centres. When two drugs are delivered as separate molecules, regulatory authorities normally require each drug to be studied alone as well as in combination. If the two are combined in a single molecule, only the single molecule needs to be studied, greatly reducing the cost of development.

Where actives other than fatty acids are present there are similar advantages. The compounds allow drugs or other compounds to be administered in the form of non-chiral relatively lipophilic compounds which release the active moieties relatively easily and are well tolerated on oral, topical or parenteral administration. Their lipophilicity enables them to be absorbed partially through the lymphatic system, so by-passing the liver, to cause less gastrointestinal irritation than with many compounds, and to facilitate transport of drugs and other agents across lipophilic barriers such as the skin, the cell membrane and the blood-brain barrier.

There is certainly evidence that very interesting specific properties in addition to ready passage of lipid barriers can be conferred on many drugs by making them more lipophilic. These properties include prolonged duration of action, reduction of side effects, bypassing of first-pass liver metabolism and, potentially, site specific delivery of different materials.

Fatty Acid Derivatives; Effects of the Fatty Acids

The transport of actives across lipid membranes may be improved by linking them directly or via intermediate links to, in particular, gamma-linolenic acid (GLA) or dihomo-gamma-linolenic acid (DGLA), two fatty acids which in themselves have a range of desirable effects. Other fatty acids, such as any of the essential fatty acids (EFAs) and in particular the twelve natural acids of the n-6 and n-3 series EFAs (fig. 1), can be used or

a) Psychotropic drugs may be linked to fatty acids such as GLA, DGLA, arachidonic acid or docosahexaenoic acid which have important roles in brain function, so providing a dual therapeutic effect.

b) Drugs used for the treatment of cardiovascular disease may be attached to a fatty acid which also has value in such treatment, such as eicosapentaenoic acid which lowers triglyceride levels and inhibits platelet aggregation, or GLA or DGLA which lower cholesterol levels and have vasodilator action, or arachidonic acid which is a potent cholesterol lowering agent, or DHA which has anti-arrhythmic properties.

c) Drugs used in the treatment of any form of inflammation may be linked to a fatty acid such as gammalinolenic acid, dihommo-gammalinolenic acid or eicosapentaenoic acid or docosahexaenoic acid which also has anti-inflammatory action.

d) Drugs used in the management of osteoporosis may be linked to GLA or DGLA which enhance the incorporation of calcium into bone, or to EPA or DHA which reduces urinary calcium excretion.

e) Drugs used in skin disease may be linked to GLA or DGLA which have anti-inflammatory effects on the skin.

f) Drugs used in cancer may be linked to GLA, DGLA arachidonic acid, EPA or DHA which have anticancer effects in their own right and which may reverse resistance to anticancer drugs.

Concepts Applied to Essential Fatty Acids as Bioactives

The essential fatty acids (EFAs) as already referred to, and well known, consist of a series of twelve compounds. Although linoleic acid, the parent compound of the n-6 series, and alpha-linolenic acid, the parent compound of the n-3 series, are the main dietary EFAs, these substances as such have relatively minor roles in the body. In order to be fully useful

adverse effects of ionising radiation, to treat various psychiatric disorders, to cause vasodilation to improve renal function, to treat the complications of diabetes, to dilate blood vessels and so on. Actives linked to GLA and DGLA will therefore not only become more lipophilic, enhancing penetration across all membranes, the skin and the blood brain barrier, but are also likely to exhibit new and additional therapeutic effects. The fatty acid compounds may thus be mutual bipartate prodrugs (if linked directly) or mutual tripartate prodrugs (if connected via a link), for example.

Other fatty acids likely to be of especial value in this context are arachidonic acid and docosahexaenoic acid which are major constituents of all cell membranes; adrenic acid; and stearidonic acid and eicosapentaenoic acid which have ranges of desirable properties similar to those of GLA and DGLA.

Classes of Actives Having Mutual Efficacy with Bioactive Fatty Acids

Kinds of actives may be broadly stated:-

a) Drugs including antibiotics, antiprotozoals, antipsychotics, antidepressants and NSAIDs.

b) Hormones

c) Amino acids

d) Vitamins particularly of the B group, and other essential nutrients.

e) Cytokines and peptides

f) Neurotransmitter and neurotransmitter precursors.

g) Phospholipid head groups such as inositol, choline, serine and ethanolamine, which may be linked directly or via the phosphate moiety.

The combination of the therapeutic effect of a drug with the therapeutic effect of a fatty acid may be considered through examples:-

molecule creates many problems in synthesis, pharmacy, pharmacology, formulation and stability. Moreover triglycerides can be slow and difficult to synthesise. When treated under similar conditions propane diol derivatives can be made much more rapidly.

For purposes of convenient administration of different fatty acids simultaneously or indeed of a single fatty acid in high amounts in well tolerated form, use is thus desirably made of esters of diols.

Chemical Nature of Bioactives which may be derivatised according to the present disclosure

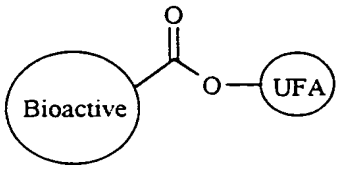
The present specification covers fatty acid (or fatty alcohol) derivatives of bioactives with an available carboxyl, alcohol or amino group such that a single, well defined chemical entity is formed. The coupling may be direct yielding bipartate compounds or spaced with an appropriate link group, yielding tripartate compounds, in terms of the number of moieties into which the compounds split.

Classes of Bioactives by Chemistry

Among classes of compounds are those below, where n is conveniently 1 to 3. The substances claimed herein are diesters have an n=3. Substances where n is a greater or lesser number, or where the links are not ester links, are likely to be of value for similar reasons and are disclosed here by not claimed.

(a) **Bioactives with a free carboxyl group**- these may be derivatised as follows:

(i) ester coupling with unsaturated fatty alcohol (UFA)



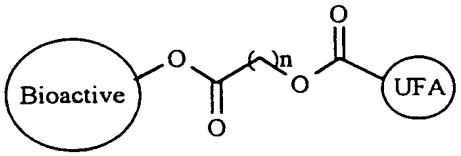
to the body, the parent compounds must be metabolised to longer chain and more highly unsaturated compounds. In quantitative terms, as judged by their levels in cell membranes and in other lipid reactions dihomogammalinolenic acid (DGLA) and arachidonic acid (AA) are the main EFA metabolites of the n-6 series while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the main metabolites of the n-3 series. DGLA, AA, EPA and DHA are important constituents of most of the lipids in the body. As well as being important in themselves they can also give rise to a wide range of oxygenated derivatives, the eicosanoids, including the prostaglandins, leukotrienes and other compounds. The fatty acids likely to be of particular value in therapy are DGLA, AA, EPA and DHA, together with GLA, the precursor of DGLA, stearidonic acid (SA), the precursor of EPA and DPA (22:5n-3), the precursor of DHA, and adrenic acid.

Further there are acids such as oleic acid, parinaric acid and columbinic acid that are not EFAs but may have significant effects in the body.

It used to be thought that, both in nutrition and in therapy of disease, it was sufficient to supply linoleic and alpha-linolenic acids and the body's own metabolism would do the rest. It is now widely accepted that this is not true. Different diseases may have different abnormal patterns of EFAs and because of problems in metabolism these cannot simply be corrected by giving linoleic or alpha-linolenic acid. It may therefore be appropriate in some situations to provide increased amounts of one of the other EFAs or to give two or more of the EFAs simultaneously. While the EFAs can be supplied in various forms and in various mixtures, it is convenient in both nutrition and in medical treatment to be able to supply the fatty acids as particular molecules.

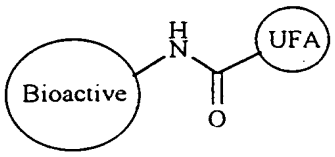
To date, proposals have been in terms of particular triglycerides, following the natural occurrence of essential fatty acids in triglyceride form. However, triglycerides, unless symmetrical about the 2-carbon, are chiral and that fact, coupled with acyl migration between the alpha and beta positions makes the synthesis of specific triglycerides a difficult task. The lack of specificity when two fatty acids are present in the same triglyceride

(iii) ester coupling with ω-carboxyalkyl ester of unsaturated fatty acid

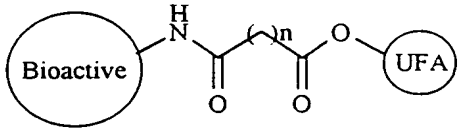


(c) **bioactives with a free amino group** - these may be derivatives as follows:-

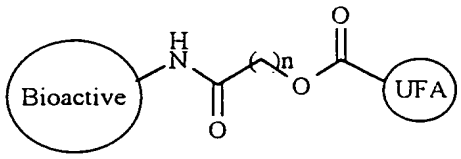
(i) amide coupling with essential fatty acid



(ii) amide coupling with ω-carboxyalkylcarboxy ester of essential fatty alcohol

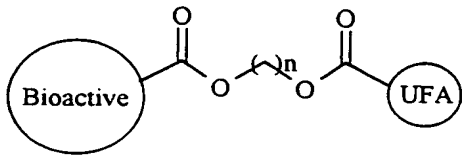


(iii) amide coupling with ω-carboxyalkyl ester of essential fatty acid

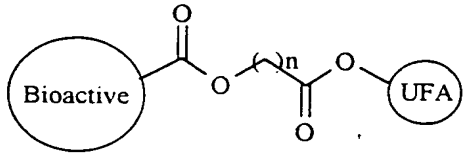


In all of the above categories, the carbon chain of the unsaturated fatty acid or alcohol is represented by:

(ii) ester coupling with ω-hydroxyalkyl ester of unsaturated fatty acid

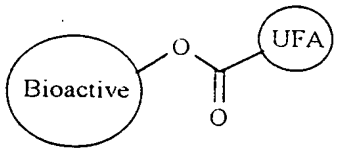


(iii) ester coupling with ω-hydroxyalkylcarboxy ester of unsaturated fatty alcohol.

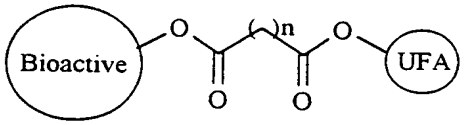


(b) bioactives with a free hydroxyl group - these may be derivatives as follows:

(i) ester coupling with unsaturated fatty acid



(ii) ester coupling with ω-carboxyalkylcarboxy ester of unsaturated fatty alcohol



(d) by reaction of alcohol with acid or acid, short or medium chain alkyl ester, or acid, activated ester, e.g. vinyl, in the presence of a hydrolase enzyme, e.g. hog liver esterase, with or without a suitable solvent, e.g. hexane, at temperatures between 20° and 80°C under conditions such that the water or alcohol or aldehyde byproduct is removed, e.g. under vacuum.

(e) by reaction of acid with suitable alcohol derivative, e.g. iodide, with or without the presence of a suitable base, e.g. potassium carbonate, in a suitable inert solvent, e.g. dimethylformamide, at a temperature between 0° and 180°C.

(f) by reaction of alcohol with acid, short or medium chain alkyl ester, in the presence of a catalytic amount of an alkoxide of type M^+OY^- where M is an alkali or alkaline earth metal, e.g. sodium, and Y is an alkyl group containing 1-4 carbon atoms which may be branched, unbranched, saturated or unsaturated, with or without the presence of a suitable solvent, e.g. toluene, at temperatures between 50° and 180°C such that the lower alcohol, HOY, is removed from the reaction mixture, e.g. under vacuum.

Derivatisation of bioactives in class (c) require the formation of an amide bond. Such chemistry may be achieved by any reasonable method of amide synthesis and especially:

(g) by reaction of amine with acid chloride, acid anhydride or suitably activated ester with or without the presence of an organic tertiary base, e.g. pyridine, in a suitable inert solvent, e.g. dichloromethane, at a temperature between 0° and 120°C.

(h) by reaction of amine with acid in the presence of a condensing agent, e.g. 1,3-dicyclohexylcarbodiimide, with or without the presence of a suitable organic tertiary base, e.g. 4-(N,N-dimethylaminopyridine), in an inert solvent, e.g. dichloromethane, at a temperature between 0° and 50°C.

(i) by reaction of amine with acid or acid, short or medium chain alkyl ester, or acid, activated ester, e.g. vinyl, in the presence of a hydrolase enzyme, e.g. hog liver esterase, with or without a suitable solvent, e.g. hexane, at temperatures between 20°and 80°C under

In all of these categories "unsaturated fatty acid" (and the derived "unsaturated fatty alcohol") represents a member of a group comprising oleic acid (and oleoyl alcohol) and any fatty acid (or corresponding fatty alcohol) with two or more *cis* or *trans* double bonds. However, the fatty acids likely to be of most value in this context are the essential fatty acids shown in fig. 1 and in particular GLA, DGLA, AA, SA, EPA and DHA.

General Discussion of Synthesis

The individual fatty acids may be purified from natural animal, vegetable or microbial sources or may be chemically synthesised by methods well known to those skilled in the art or by methods to be developed in the future.

The individual fatty alcohols may be prepared by chemical reduction of the fatty acids outlined above by methods well known to those skilled in the art or by methods to be developed in the future.

Derivatisation of bioactives in classes (a), (b) and (c)[subclasses (ii) and (iii)] requires the formation of one or more ester bonds. Such chemistry may be achieved by any reasonable method of ester synthesis and especially:

(a) by reaction of alcohol with acid chloride, acid anhydride or suitably activated ester with or without the presence of an organic tertiary base, e.g. pyridine, in a suitable inert solvent, e.g. dichloromethane, at a temperature between 0° and 120°C.

(b) by reaction of alcohol with acid or acid, short or medium chain alkyl ester, in the presence of a suitable acid catalyst, e.g. 4-toluene sulfonic acid, with or without a suitable inert solvent, e.g. toluene, at a temperature between 50° and 180°C such that the water formed in the reaction is removed, e.g. under vacuum.

(c) by reaction of alcohol with acid in the presence of a condensing agent, e.g. 1,3-dicyclohexylcarbodiimide, with or without the presence of a suitable organic tertiary base, e.g. 4-(N,N-dimethylaminopyridine), in an inert solvent, e.g. dichloromethane, at a temperature between 0° and 50°C.

Anti-oxidants

GLA-lipoic acid, DHA-lipoic acid, GLA-tocopherol, di-GLA-3,3'-thiodipropionic acid and in general any of GLA, DGLA, AA, SA, EPA or DHA with any natural or synthetic anti-oxidant.

Drugs

GLA and indomethacin, ibuprofen, fluoxetine, ampicillin, penicillin V, sulindac, salicylic acid, metronidazole, fluphenazine, dapsone, tranylcypromine, acetyl carnitine, haloperidol, mepacrine, chloroquine, penicillin, tetracycyline, pravastatin and agents used as x-ray contrast media, and in general any of GLA, DGLA, AA, SA, EPA or DHA with any drug.

Concepts Applied to NSAIDs; Effectiveness Shown

As a particular example of the concepts discussed, we have prepared derivatives of various non-steroidal anti-inflammatory drugs (NSAIDs) and in particular the GLA-ester of indomethacin. Indomethacin as a non-steroidal anti-inflammatory drug is believed to have a primarily intracellular mechanism of action by inhibiting the enzyme cyclo-oxygenase, which converts arachidonic acid to pro-inflammatory prostaglandin metabolites.

Indomethacin is known to penetrate cells very poorly and so has to be given in relatively large doses which can produce many side effects, thus indomethacin-GLA was compared with indomethacin itself for its ability to penetrate cells, using a normal fibroblast line, a breast cancer line and a malignant melanoma line.

The results are set out in EPA-0675103 and show that in all the cell lines the intracellular level of indomethacin after incubation with indomethacin was very low and mainly detected only in trace amounts. In contrast, again in all cell lines, incubation with indomethacin-GLA resulted in very substantial amounts of both indomethacin-GLA and free

conditions such that the water or alcohol or aldehyde byproduct is removed, e.g. under vacuum.

Examples of Pairs of Actives which may be linked either directly or via a link, particularly a Propane Diol link

Examples of pairs of actives follow, all the compounds listed being, to our knowledge, entirely novel compounds. We therefore claim them as new chemical entities as well as their use in treatment or prevention of disease.

Fatty Acids

GLA-OA (OA = Oleic Acid), GLA-GLA, EPA-EPA, GLA-EPA, GLA-DHA, AA-DHA, AA-EPA, GLA-AA, GLA-SA, SA-DHA, AA-SA, DGLA-DGLA, DGLA-GLA, DGLA-SA, DGLA-AA, DGLA-EPA, DGLA-DHA.

Vitamins

GLA-niacin, GLA-retinoic acid, GLA-retinol, GLA-pyridoxal, Di-GLA-pyridoxine, EPA-pyridoxine an in general either GLA, DGLA, AA, SA, EPA or DHA with any vitamin.

Amino acids

GLA-tryptophan, GLA-proline, GLA-arginine, GLA- or DHA-phenylalanine GLA-GABA, GLA-aminolevulinic acid and in general either GLA, DGLA, AA, SA, EPA or DHA with any natural amino acid.

Steroids

GLA-hydrocortisone, GLA-oestradiol, GLA- and DHA-dehydroepiandrosterone and in general any of GLA, DGLA, AA, SA, EPA or DHA with any natural or synthetic steroid.

isomers. We have for example shown that intravenous infusion and oral administration GLA/EPA diol ester emulsion leads to rapid in vivo release of free GLA and EPA and to further metabolism of the GLA to AA and of the EPA to DHA. Similarly, GLA-GLA and EPA-EPA diols, and niacin-GLA and indomethacin-GLA diols have been shown to be absorbed following oral administration and to release their active moieties.

The fatty acid diesters have a wide variety of possible uses. They may be used as pharmaceuticals for the treatment or prevention of diseases in which abnormalities of fatty acids have been identified. They may be added to foods or added to or used as nutritional supplements for those who require the particular fatty acid for the treatment or prevention of diseases. They may also be used in foods or pharmaceuticals for veterinary use. They may further be used for skin care.

In various particular aspects besides those in the claims herein, the invention provides:

(i) A convenient and safe way of administering, for therapeutic or nutritional purposes, one or two unsaturated fatty acid moieties or one unsaturated fatty acid and one non essential fatty acid bioactive.

(ii) A derivative of a bioactive required to cross lipid membranes in the body to exert its action whether in entry to a cell or in passing the skin, blood-brain or other barrier, through a 1,3-propane diol linkage to an essential fatty acid of the natural n-6 or n-3 series and especially GLA or DGLA, AA, SA, EPA or DHA.

(iii) A fatty acid derivative of a drug such that the drug and fatty acid are mutually efficacious.

(iv) A method of improving the transport of a drug across lipid membranes in the body, characterised by the administration of the drug in a form as above.

(v) A method of manufacture of a medicament for improved therapy involving transport of a drug across lipid membranes in the body, characterised by incorporating the drug in a medicament in a form as above.

indomethacin being found within the cells. These results show unequivocally that the GLA ester of indomethacin penetrates cells effectively and is then de-esterified intracellularly to provide fee indomethacin, and that in view of the many similarities between the cell membrane barrier and the blood-brain and skin barriers, the indomethacin-GLA will also be effective in accelerating the penetration of indomethacin through these barriers.

The Present Invention

Aspects of the invention are set out in the claims herein, but it is also discused broadly below.

While direct linkages of bioactives and fatty acids (classes (a)[i], (b)[i] and (c)[i] are discussed above, the present invention concerns class (a)[ii], n=3 whereby bioactives, which may themselves be fatty acids, are linked to fatty acids as diesters of 1,3-propane diol. This diol may also be regarded as 2-deoxyglycerol. The compounds listed herein are almost all new chemical entities and where they are not new such chemical entities have never previously been used in treatment of human or animal disease.

As a diol the link is, broadly, disclosed in the literature among many other diols but we have seen that its use in therapy in the form of an essential fatty acid diester or as a compound with an essential fatty acid at one position and a bioactive (not being an essential fatty acid) at the other, is both undisclosed and particularly significant. Indeed it offers a favourable way to give a single fatty acid as the monester or diester if a completely defined compound is required, as there is no chiral centre such as is present in glycerol monoesters if a terminal carbon is involved and in diesters if the central carbon is involved, nor do positional isomers exist. Further, apart from administering individual acids, such mono and diesters may have value in pharmaceutical formulation as emulsifiers. The 1,3-propane diol structure is close to the glycerol of natural triglycerides and an effective and safe delivery system. Moreover it allows ready and unequivocal synthesis of defined compounds without the problems of acyl migration shown in triglycerides and without complications by optical

monitored carefully. This method also suffers from the fact that the fatty acid chlorides themselves must first be manufactured; this additional step reduces the overall efficiency the process. A particular family of enzymes, the lipases, can be used to catalyse the esterification reaction under very mild conditions (e.g. at 60°C), and are probably the catalysts of choice when polyunsaturated fatty acids are being used. However, most enzymes interact most effectively with the 1- and 3- positions of glycerol. Addition of fatty acid to the 2- position is slow, and often dependant upon "acyl migration", i.e. a fatty acid must first be attached to the 1- or 3- position, and then migrate to the 2- position, where it remains attached. Thus, triglyceride synthesis reactions which are catalysed by enzymes can take days to approach completion (see Fig. 2).

In theory, the same methods can be applied to the esterification of 1,3-propandiol as can be applied to glycerol. However, when it is considered that enzymes catalyse preferentially the addition of fatty acids to the 1- and 3- positions of glycerol, it is clear they should be particularly effective when used to make di-esters. This is indeed the case, with reactions being completed in a matter of hours and at temperatures which are even lower (e.g. 45°C to 60°C) than those required for triglyceride synthesis (see Fig.3). After four hours free fatty acid can be absent, and after eight hours the yield of di-ester can be in excess of 95%, the balance being monoester.

A further complexity with specific triglyceride syntheses is the presence within glycerol of both primary and secondary hydroxyl groups and a prochiral centre at the central carbon atom. These problems may be solved by the use of carefully selected protecting groups and by chiral synthesis. However, this results in multistep syntheses with decreasing yield and increasing impurity levels at each step. In contrast, however, 1,3-propane diol possesses only primary hydroxyl groups and no prochiral centres. The synthesis is consequently reduced to two steps maximum with improved overall yield and decreased impurity levels.

Examples of specific compounds have been given earlier herein; synthesis examples come later.

EASE OF SYNTHESIS

Synthesis of Triglycerides

The following considers the advantages of use of 1,3-propane diol compared in particular to triglycerides.

Specifically, it is proposed that 1,3-propandiol be used in place of glycerol in the esterification of fatty acids, especially where only one type of fatty acid (e.g. gamma-linolenic acid) is to be attached to the three-carbon chain "backbone". Although diesters and triglycerides are chemically very similar, the manufacture of di-esters can be carried out under very mild conditions, and in a matter of hours. To manufacture triglycerides, either harsh conditions are required, or fatty acid chlorides must be used, or biocatalysts (which require reaction times of several days) are necessary.

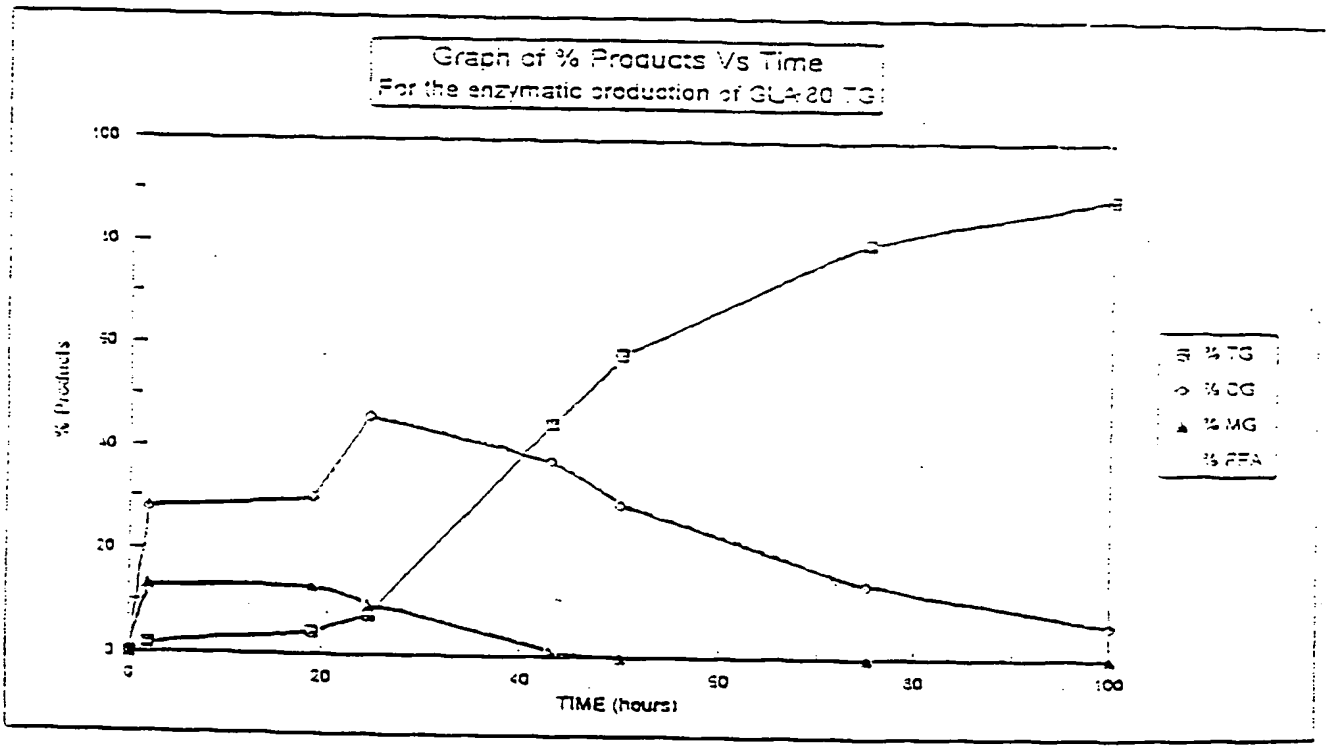
A summary of triglyceride synthesis methods is:

1. Chemical reaction with metals, metal-chlorides, or organic acids as catalyst.
2. Use of fatty-acid chlorides.
3. Use of immobilised enzymes.

All processes using acids, metals, or metal chlorides as catalysts are very similar and share a common list of advantages and disadvantages. Many of the problems are inherent to the methods, i.e. acidic conditions and high temperatures (140°C to 180°C). The p-TSA method probably exhibits the least problems, as this is carried out under the mildest conditions (140°C). Reaction of glycerol with fatty acid chlorides is done under "cold" conditions, but toxic gases are evolved and the reaction can go out of control if not

FIGURE 2: TRIGLYCERIDE SYNTHESIS:

TIME (hours)	% TG	% DG	% MG	% FFA
0	0	0	0	100
2	1.74	27.9	13.11	57.25
19	4.25	30.15	12.93	52.68
24.5	7.41	46.13	9.48	36.98
43	44.84	37.55	0.84	16.78
50	58.36	29.19	0	12.46
75	80.23	13.86	0	5.9
100	89.1	6.17	0	4.73



In summary, the reaction which prepares di-esters from polyunsaturated fatty acids and 1,3-propandiol is faster, and can be carried out under much milder conditions, than can the corresponding triglyceride synthesis. This leads to a more economical and less wasteful production process and minimises the risk of reactants or products becoming altered or degraded during processing.

Formulations

The compounds may be formulated in any way appropriate and which is known to those skilled in the art of preparing pharmaceuticals, skin care products or foods. They may be administered orally, enterally, topically, parenterally (subcutaneously, intramuscularly, intravenously), rectally, vaginally or by any other appropriate route.

The doses of the actives to be administered largely range from 1mg to 100g per day, preferably 10 mg to 10 g and very preferably 10mg to 3g, according to their kind. They may be administered topically in preparations where the actives form from 0.001% to 50% of the topical preparation, preferably 0.05% to 20% and very preferably 0.1% to 10%.

Examples

Illustrative syntheses of NSAID's linked to fatty acids are given in EPA-0675103 referred to earlier. Illustrative syntheses of the linking of fatty acids, through 1,3-propane diol residues follow, with other generally illustrative material.

Example 1

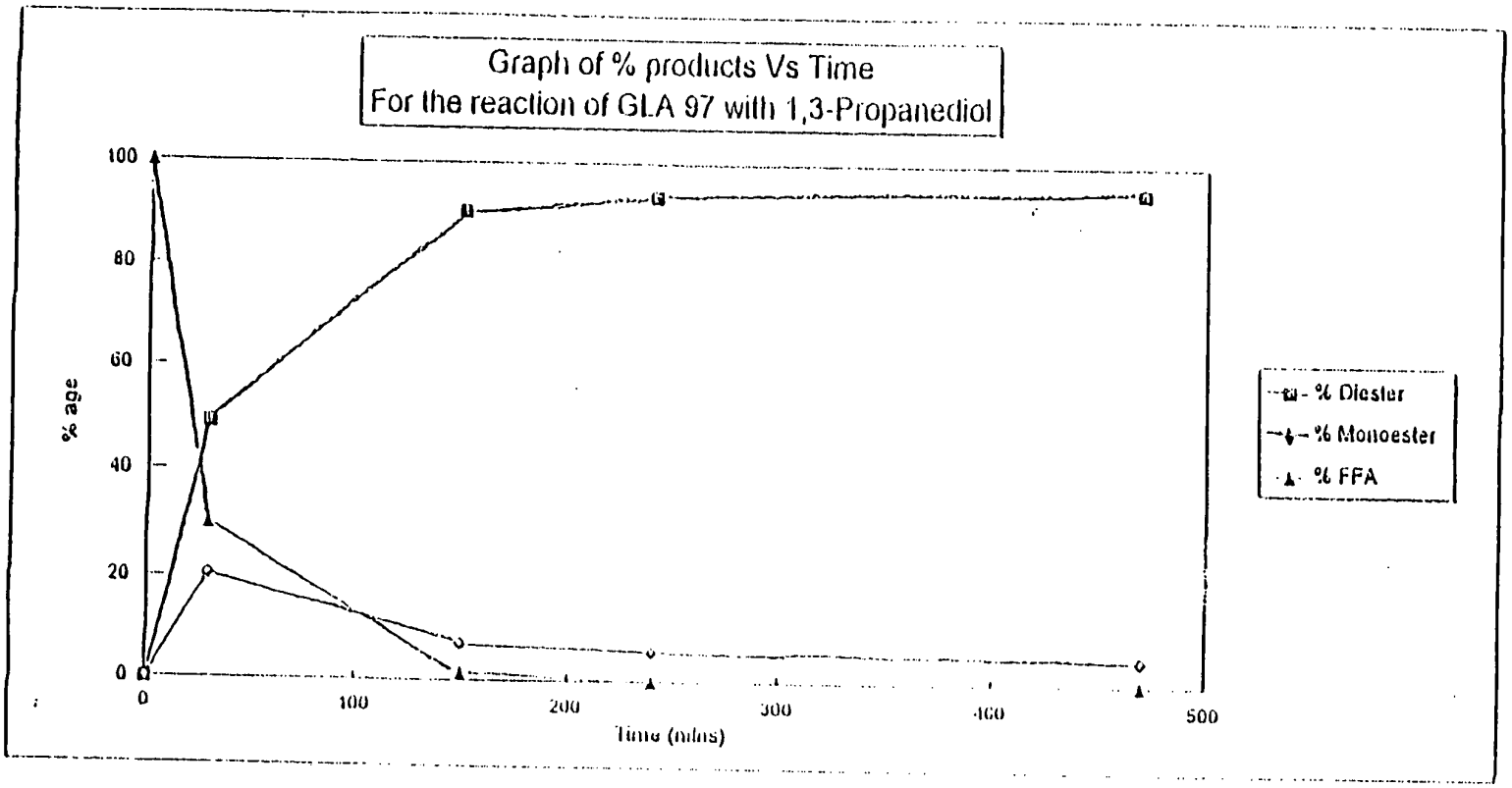
z,z,z-octadeca-6,9,12-trienoyl-1- (4-chlorobenzoyl)-5-methoxy-2-methyl indole-3-acetate *(Ester of indomethacin with GLA alcohol)*

A solution of indomethacin (50.4g) and thionyl chloride (33.3 ml) in 1,2-dichloroethane (700ml) was heated at 90°C under nitrogen for 4 hours. The solvent was removed *in vacuo* and further portions of 1,2-dichloroethane (2 x 200 ml) were added and evaporated to remove the last traces of thionyl chloride. To a solution of the dark, solid residue in dichloromethane (700 ml) was added pyridine (11.7 g) and z,z,z-octadeca-6,9,12-trienol (35.4 g). The mixture was stirred under nitrogen at room temperature overnight. The mixture was then concentrated to dryness under reduced pressure and diluted with ethyl

Figure 3: Di-ester Synthesis.

Graph of % Products vs Time for the enzymatic production of GLA diester

Time (mins)	% Diester	% Monoester	% FFA
0	0	0	100
30	49.01	20.83	30.16
150	90.7	7.37	1.48
240	93.63	5.89	0
470	95.08	4.92	0



Example 4

z,z,z-octadeca-6,9,12-trienyl-(Z)-5-fluoro-2- methyl-1- [[4-(methylsulfinyl) phenyl]methylene]- lH-indene-3-acetate.

(Ester of sulindac with GLA alcohol)

In a similar manner but replacing the indomethacin with the requisite amount of (Z)-5-fluoro-2-methyl-1-[[4-(methylsulfinyl)phenyl]methylene]- 1 H-indene-3-acetic acid (sulindac) and the z,z,z-eicosa-8,11,14-trienol with z,z,z-octadeca-6,9,12-trienol there is prepared z,z,z-octadeca-6,9,12-trienyl-(Z)-5-fluoro-2-methyl-1- [[4-methylsulfinyl)phenyl]methylene]- lH-indene-3-acetate.

Example 5

2-(z,z,z-octadeca-6,9, 12-trienoyl)benzoic acid.

(GLA ester of salicylic acid).

Part 1.

To a solution of 2,2,2-trichloroethyl salicylate (104g) in dry pyridine (500 ml) at 0-5°C and under nitrogen was added z,z,z-octadeca-6,9,12-trienoyl chloride (137.5g) dropwise over a period of 2 hours. The reaction mixture was allowed to stir at room temperature overnight and the pyridine was then removed *in vacuo*. The residue was dissolved in diethyl ether (2000 ml) and water (1000ml) and the resulting two phase system was shaken and acidified slowly to pH1 by addition of 2M hydrochloric acid. The diethyl ether layer was separated and washed with water (4 x 1000 ml), adding sodium chloride to break any emulsion that formed. After drying the organic layer (sodium sulfate), the solvent was removed *in vacuo* to give an orange/brown oil. This was purified by flash chromatography to give 2,2,2-trichloroethyl-2-(z,z,z-octadeca-6,9,12-trienoyl) benzoate as a pale yellow oil.

acetate. The organic layer was successively washed with brine, 2M hydrochloric acid, saturated aqueous sodium bicarbonate, and water. After drying (sodium sulfate), the solvent was evaporated to give a yellow oil which was purified by flash chromatography giving z,z.z-octadeca-6,9,12-trienoyl-1-(4-chlorobenzoyl)-5-methoxy-2-methyl indole-3-acetate as a yellow oil.

Example 2

z,z,z-eicosa-8,11,14- trienoyl-1- (4-chlorobenzoyl)-5-methoxy-2-methyl indole-3-acetate

(Ester of indomethacin with DGLA alcohol)

A solution of indomethacin (36.7g) and 4-(N,N-dimethylamino) pyridine (12.5 g) in dichloromethane (100ml) was added dropwise, with stirring, over the course of 30 minutes, under nitrogen, to a mixture of 1,3-dicyclohexyl carbodiimide (21.2 g) and z,z,z-eicosa-8,11,14-trienol (27.3g) in dichloromethane (400ml). Stirring was continued for 5 hours. The reaction was filtered, concentrated to dryness *in vacuo* and purified by flash chromatography giving *z,z,z*-eicosa-8,11, 14-trienyl-1-(4-chlorobenzoyl)-5-methoxy-2-methyl indole-3-acetate as a pale yellow oil.

Example 3

z,z,z-octadeca-6,9,12-trienyl-2-methyl-4'-(2-methylpropyl)-phenylacetate.

(Ester of ibuprofen with GLA alcohol)

In a similar manner but replacing the indomethacin with the requisite amount of 2-methyl-4'-(2-methylpropyl)-phenylacetic acid (ibuprofen) and the z,z,z-eicosa-8,11,14-trienol with z,z,z-octadeca-6,9,12-trienol there is prepared *z,z,z*-octadeca-6,9,12-trienyl-2-methyl-4'-(2-methylpropyl)-phenyl acetate.

In a similar manner but replacing the linoleic acid with the requisite amount of z,z,z,z,z,z-docosa-4,7,10,13,16,19-hexaenoic acid there is prepared 2-(2-methyl-5-nitroimidazoloyl)ethyl-z,z,z,z,z,z-docosa-4,7,10,13,16,19-hexaenoate.

Example 10

4-[3-[2- (trifluoromethyl) 10H-phenothiazin-10-yl]]-1-piperazineethyl-z,z,z-octadeca-6,9,12-trienoate

(Ester of fluphenazine with GLA)

In a similar manner but replacing the metronidazole with the requisite amount of the free base of4-[3-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]]-1-piperazineethanol (fluphenazine) and the linoleic acid with the requisite amount of GLA there is prepared 4-[3-[2-(trifluoromethyl)-10H-phenothiazin- 10-yl]]- 1-piperazineethyl-z,z,z-octadeca-6,9,12-trienoate.

Example 11

4,4'-(bis z,z,z-octadeca-6,9,12-trienoylamino)diphenylsulfone.

(Bis amide of dapsone with GLA)

In a similar manner but replacing the metronidazole with the requisite amount of 4,4'-diamino diphenylsulfone (dapsone) and the linoleic acid with the requisite amount of GLA there is prepared 4,4'-(bis z,z,z-octadeca-6,9,12-trienoylamino)diphenylsulfone.

Example 12

trans- 1-*(z,z,z*-octadeca-6,9,12-trienoylamino)-2-phenyl cyclopropane.

(Amide of tranylcypromine with GLA)

In a similar manner but replacing the metronidazole with the requisite amount of *trans*-1-amino-2-phenylcyclopropane (tranylcypromine) and the linoleic acid with the requisite

Part 2.

2,2,2-Trichloroethyl-2-(z,z,z-octadeca-6,9, 12-trienoyl)benzoate (151g) was dissolved in a mixture of tetrahydrofuran (750 ml), acetic acid (675 ml) and water (75ml). Zinc dust (150 g) was added. The mixture was stirred at room temperature under nitrogen for 2 hours and then allowed to stand overnight. Excess zinc and zinc salts were filtered off through Celite, washing the filter pad with tetrahydrofuran (100 ml) and the filtrate was evaporated at 25°C, 0.5 mm Hg. The resulting oil was dissolved in diethyl ether (1000 ml) and the resulting solution was washed with water. After drying (sodium sulfate), stirred at room temperature overnight. To the reaction was added 2M hydrochloric acid (20 ml) and stirring was continued. After filtration the organic layer was separated, washed with 50% saturated brine and finally with saturated aqueous sodium bicarbonate. The dichloromethane solution was dried (sodium sulfate) and evaporated *in vacuo* (30°C/20mm Hg). To the resulting residue was added petrol (bp 30 - 60°C, 20ml) and the mixture allowed to stand at room temperature for 2 hours, causing the precipitation of the remaining urea. This was removed by filtration and the filtrate was applied to a dry column giving 2-(2-methyl-5-nitroimidazolyl) ethyl-z,z-octadeca-9,12-dienoate as a pale yellow, non distillable oil.

Example 8

2-(2-methyl-5-nitroimidazoloyl) ethyl-z,z,z-eicosa-8,11,14-trienoate.

(Ester of metronidazole with DGLA)

In a similar manner but replacing the linoleic acid with the requisite amount of z,z,z-eicosa-8, 11, 14-trienoic acid there is prepared 2-(2-methyl-5-nitroimidazoloyl) ethyl-z,z,z-eicosa-8, 11, 14-trienoate.

Example 9

2-(2-methyl-5-nitroimidazoloyl)ethyl-z,z,z,z,z,z-docosa-4,7,10,13,16,19- hexaenoate

(Ester of metronidazole with DHA)

Example 15

1 ,3-(di-*z,z,z*-octadeca-6,9,12-trienoyloxy)propane.

(Diester of GLA with 1,3-propane diol)

A solution of 1,3-dicyclohexylcarbodiimide (1.07g) and 4-(N,N-dimethylamino)pyridine (0.59g) in methylene chloride (5ml) was added to a solution of 1,3-dihydroxypropane (0. 152ml) and *z,z,z*-octadeca-6,9,12-trienoic acid (95%, 1.36g) in methylene chloride (15ml). The reaction was stirred at room temperature under nitrogen until it was complete as determined by tlc. Hexane (80ml) was added to the reaction. The precipitate was removed by filtration and washed thoroughly with hexane. The combined filtrates were concentrated and purified by flash chromatography to yield 1,3-(di-z,z,z-octadeca-6,9,12-trienoyloxy)propane as a pale yellow free flowing oil.

Example 16

1-(z,z,z-octadeca-6,9, 12-trienoyloxy)-3-(z-octadec-9-enoyloxy)propane.

(Diester of GLA and oleic acid with 1,3-propane diol).

Part 1:

A solution of z,z,z-octadeca-6,9,12-trienoic acid (150g) in methylene chloride (500ml) was added dropwise to a mixture of 1,3-dihydroxypropane (205g), 1,3-dicyclohexylcarbodiimide (130g) and 4-(N,N-dimethylamino)pyridine (87g) in methylene chloride (2500ml) at room temperature under nitrogen. When tlc indicated that the reaction had gone to completion the reaction mixture was filtered. The filtrate was washed with dilute hydrochloric acid, water and saturated sodium chloride solution. The solution was dried, concentrated and purified by dry column chromatography to yield 1-*(z,z,z*-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane as a pale yellow oil.

Part 2:

A solution of 1,3-dicyclohexylcarbodiimide (23.7g) and 4-(N,N-

amount of GLA there is prepared *trans-1-(z,z,z*-octadeca-6,9,12-trienoylamino)-2-phenyl cyclopropane.

Example 13

N-methyl-3-phenyl -3[α,α,α-trifluoro-p-tolyl] propyl; *z,z,z*-octadeca-6,9,12-trienamide *(Amide of fluoxetine with GLA)*

In a similar manner but replacing the metronidazole with the requisite amount of N-methyl-3-phenyl-3[α,α,α-trifluoro-p-tolyl]propylamine (fluoxetine) and the linoleic acid with the requisite amount of GLA there is prepared N-methyl-3-phenyl -3[α,α,α-trifluoro-p-tolyl] propyl-z,z,z-octadeca-6,9,12-trienamide.

Example 14

6-[(aminophenylacetyl)amino]-3 ,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid-z,z,z-octadeca-6,9,12-trienamide

(Amide of ampicillin with GLA)

Triethylamine (0.3 ml) was added to a stirred suspension of ampicillin (0.7 g) in anhydrous DMF (120m1) under a nitrogen atmosphere. To the resultant clear solution was added z,z,z-octadeca-6,9,12-trienoic acid, N-hydroxysuccinimide ester (0.75 g) while maintaining the reaction at 0-10°C. The reaction was stirred at this temperature for an additional hour before allowing the mixture to stand at room temperature overnight. Tlc analysis (40% THF/hexane) at this point indicated that most of the succinimide ester had reacted. Water (40ml) was added to the reaction flask and the contents stirred. The solution was then neutralised and extracted with ethyl acetate. The extract was washed with water, dried (sodium sulfate) and concentrated to dryness leaving the crude product as a yellow glass. Trituration with hexane yielded 6-[(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-4-thia -1-azabicyclo[3.2.0] heptane-2-carboxylic acid-z,z,z-octadeca-6,9,12-trienamide as a yellow powder.

(Diester of GLA and EPA with 1,5-pentane diol).

Part 1:

z,z,z-Octadeca-6,9,12-trienoyl chloride (2 g) was added dropwise to a solution of 1,5-dihydroxypentane (3.5g), triethylamine (0.94ml) and 4-(N,N-dimethylamino)pyridine (0.2g) in methylene chloride (50ml) with stirring at 0°C under nitrogen. On completion of reaction as evidenced by tlc the reaction mixture was washed with dilute hydrochloric acid and water, dried and purified by column chromatography yielding 1-*(z,z,z*-octadeca-6,9,12-trienoyloxy)-5-hydroxypentane as a pale yellow oil.

Part 2:

As for Example 16, Part 2 but replacing 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane with 1-(z,z,z-octadeca-6,9, 12-trienoyloxy)-5-hydroxypentane and z-octadeca-9-enoic acid with *z,z,z,z,z-eicosa-5,8,11,14,17*-pentaenoic acid. Chromatography yielded 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-5-(z,z,z,z,z-eicosa-5,8, 11,14,17-pentaenoyloxy)pentane as a pale yellow oil.

Example 20

1-(z,z,z-octadeca-6,9,12-trienoyloxy)-4-(z,z,z,z,z-eicosa-5 ,8, 11,14,17-pentaenoyloxy)benzene.

(Diester of GLA and EPA with 1,4-dihydroxybenzene).

Prepared as in Example 19, Parts 1 and 2 but replacing 1,5-dihydroxypentane with 1,4-dihydroxybenzene in Part 1 and replacing methylene chloride with tetrahydrofuran as the solvent in Part 1. Chromatography yielded 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-4-(z,z,z,z,z-eicosa-5,8, 11,14, 17-pentaenoyloxy)benzene as a pale yellow oil.

Example 21

z,z,z-octadeca-6,9,12-trienyl-z,z,z-octadeca-6,9,12-trienoate.

(Ester of GLA with GLA alcohol)

dimethylamino)pyridine (15.9g) in methylene chloride (200ml) was added to a solution of 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-hydroxypropane (33 .6g) and z-octadec-9-enoic acid (30g) in methylene chloride (400ml) under nitrogen at room temperature. On completion of reaction as evidenced by tlc analysis the solution was diluted with hexane, filtered, concentrated and purified by dry column chromatography to yield 1-(z,z,z-octadeca-6,9,12-trienoyloxy)-3-(z-octadec-9-enoyloxy)propane as a free flowing pale yellow oil.

Example 17

1-*(z,z,z*-octadeca-6,9,12-trienoyloxy)-3-*(z,z,z,z,z*-eicosa-5,8,11,14,17-pentaenoyloxy)propane.

(Diester of GLA and EPA with 1,3-propane diol).

Prepared as in Example 16, Part 2 but replacing z-octadeca-9-enoic acid with z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoic acid. Chromatography yielded 1-*(z,z,z*-octadeca-6,9,12-trienoyloxy)-3-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyloxy)propane as a pale yellow oil.

Example 18

1,3-di(z,z,z-octadeca-6,9,12-trienoyloxy)propane.

(Diester of GLA with 1,3-propane diol).

Prepared as in Example 16, Part 2 but replacing z-octadeca-9-enoic acid with z,z,z-octadeca-6,9,12-trienoic acid. Chromatography yielded 1,3-(di-*z,z,z*-octadeca-6,9,12-trienoyloxy)propane as a pale yellow oil.

Example 19

1-(z,z,z-octadeca-6,9,12-trienoyloxy)-5-(z,z,z,z,z-eicosa-5,8,11,14,17-pentaenoyloxy)pentane.

A solution of 1,3-dicyclohexylcarbodiimide (0.83 parts, g) and 4-(N,N-dimethylamino)pyridine (0.55 parts, g) in methylene chloride (20 parts, ml) was added . solution of 1-(z,z,z-octadeca-6,9,12-trienyl)-butane-1,4-dioate (1.32 parts, g) and z,z,z-octadeca-6,9,12-trienol (0.98 parts, g) in methylene chloride (40 parts, ml). On completion, as evidenced by tlc analysis, the reaction mixture was diluted with hexane, filtered, concentrated and purified by chromatography to yield 1,4-di(z,z,z-octadeca-6,9,12-trienyl)-butane-1,4-dioate as a pale yellow oil.

Example 24

(±)-1-(1,2-dithiolane-3-pentanoyloxy)-3-(z,z,z-octadeca-6,9,12-trienoyloxy)propane.

(Diester of lipoic acid and GLA with 1,3-propane diol)

A mixture of 1,3-dicyclohexylcarbodiimide (720mg, 3.45mmol) and 4-(N,N-dimethylamino)pyridine (480mg, 3.98mmol) in tert-butyl methyl ether (15ml) was added to a mixture of lipoic acid (645mg, 3.12mmol) and *3-(z,z,z*-octadeca-6,9,12-trienoyloxy)propan-1-ol (1g, 3mmol) in *tert*-butyl methyl ether (30ml). The mixture was stirred at room temperature under nitrogen for 5h, the progress of reaction being monitored by tlc (40% ethyl acetate/hexane). On completion the mixture was filtered, concentrated and purified by flash chromatography (hexane, 2% ethyl acetate/hexane, 5% ethyl acetate/hexane and finally 10% ethyl acetate/hexane) to yield (±)-1-(1,2-dithiolane-3-pentanoyloxy)-3-(z,z,z-octadeca-6,9,12-trienoyloxy) propane as a viscous yellow oil.

Example 25

1-([Z]-5-fluoro-2-methyl-1-[4-{methylsulfinyl}benzylidene]indene-3-acetyloxy)-3-(z,z,z-octadeca-6,9,12-trienyloxy)propane.

(Diester of sulindac and GLA with 1,3-propane diol)

1,3-dicyclohexylcarbodiimide (0. 82g) and 4-(N,N-dimethylamino)pyridine (0.48g) in methylene chloride (5ml) were added to a solution of z,z,z-octadeca-6,9,12-trienol (0.95g) and z,z,z-octadeca-6,9,12-trienoic acid (1g) in methylene chloride (10ml) with stirring at room temperature under nitrogen. On completion of reaction as evidenced by tlc, hexane was added to the reaction mixture which was subsequently filtered and purified by column chromatography to yield z,z,z-octadeca-6,9,12-trienyl-z,z,z-octadeca-6,9,12-trienoate as a pale yellow oil.

Example 22

z,z,z-octadeca-6,9,12-trienyl-z,z,z,z,z-eicosa-5,8,11,14, 17-pentaenoate.

(Ester of EPA with GLA alcohol).

Prepared as in Example 21 but replacing z,z,z-octadeca-6,9,12-trienoic acid with z,z,z,z,z-eicosa-5,8,11,14, 17-pentaenoic acid.

Example 23

1,4-di(z,z,z-octadeca-6,9,12-trienyl)-butane-1,4-dioate.

(Diester of GLA alcohol with succinic acid)

Part 1:

A solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (0.54 parts, ml) in dry tetrahydrofuran (10 parts, ml) was added dropwise to a cooled (0°C) solution of z,z,z-octadeca-6,9,12-trienol (1 part, g) and succinic anhydride (0.36 parts, g) in dry tetrahydrofuran (20 parts, ml). On completion of reaction as evidenced by tlc, the reaction mixture was diluted with diethyl ether and washed with dilute hydrochloric acid, water and brine. The organic layer was dried, concentrated and used directly in the second part of the reaction.

Part 2:

centrifuged. The hexane washing procedure was carried out once more to yield 1-([R]-3-acetoxy-4-[trimethylammonio]butyroyloxy)-3-(z,z,z-octadeca-6,9,12-trienoyloxy)propa.

Example 27

1-(3,3-dimethyl-7-oxo-6-([phenoxyacetyl)amino]-4-thia-1-azabicyclo [3.2.0]heptan-2-oyloxy)-3-(z,z,z-octadeca-6,9,12-trienoyloxy)propane.

(Diester of penicillin V and GLA with 1,3-propane diol).

A mixture of penicillin V (1g, 2.9mmol), 3-(z,z,z-octadeca-6,9,12-trienoyloxy)propan-1-ol (860mg, 2 .6mmol), 1 ,3-dicyclohexylcarbodiimide (620mg, 3mmol) and 4-(N,N-dimethylamino)pyridine (catalytic amount) in dichloromethane (30ml) was stirred overnight at room temperature. The reaction mixture was diluted with hexane (50ml), filtered and concentrated to dryness. The residue was washed with hexane (3x50ml) to remove unreacted 3-(z,z,z-octadeca-6,9,12-trienoyloxy)propan-1-ol. The semisolid residue was disolved in diethyl ether (150ml), washed with water (100ml) and dried. The ether solution was diluted with hexane (125ml) and the solution filtered through a bed of silica (4cm x 4cm). The filtrate was concentrated, yielding 1-(3 ,3-dimethyl-7-oxo-6-([phenoxy acetyl)amino]-4-thia-1-azabicyclo [3.2.0]heptan-2-oyloxy)-3-(z,z,z-octadeca-6,9,12-trienoyloxy)propane as a viscous colourless oil.

A solution of 1,3-dicyclohexylcarbodiimide (720mg, 3.45mmol) in *tert* butyl methyl ether (30ml) was added to a mixture of sulindac (1.12g, 3.15mmol), 4-(N,N-dimethylamino)pyridine (480mg, 3 .9mmol) and 3-(z,z,z-octadeca-6,9,12-trienoyloxy)propan-1-ol (1g, 3mmol) in tert-butyl methyl ether (15ml). The mixture was stirred at room temperature under nitrogen for 5h, the progress of reaction being monitored by tlc (40% ethyl acetate/hexane). On completion the mixture was filtered, concentrated and purified by flash chromatography (40% ethyl acetate/hexane, then 50% ethyl acetate/hexane and finally 60% ethyl acetate/hexane) to yield 1-([Z]-5-fluoro-2-methyl-1-[4-{methylsulfinyl}benzylidene]indene-3-acetyloxy)-3-(z,z,z-octadeca-6,9,12-trienyloxy)propane as a waxy yellow solid.

Example 26

1-([R]-3-acetoxy-4-[trimethylammonio]butyroyloxy)-3-(z,z,z-octadeca-6,9,12-trienoyloxy)propane.

(Diester of acetyl carnitine and GLA with 1,3-propane diol).

Freshly distilled thionyl chloride (1.5ml) was slowly added to (R)-acetyl carnitine (1g) in a pear shaped flask. Care was taken to contain the reagents at the bottom of the flask until a clear solution resulted. After 4 hours at room temperature excess thionyl chloride was removed under reduced pressure (keeping the flask temperature less than 30°C). This yielded the acid chloride as a highly hygroscopic white solid which was used immediately without further purification. To the flask were added 3-(z,z,z-octadeca-6,9,12-trienoyloxy)propan-1-ol (1.4g, 4.17mmol) and dry THF (4ml). The mixture was allowed to stand overnight at room temperature. Tlc analysis (40% ethyl acetate/hexane) indicated that the reaction had gone to completion. The reaction mixture was added dropwise to hexane (250ml) with vigorous stirring. A fine off white precipitate formed which was collected by centrifugation. On removal of the supernatant the solid was resuspended in hexane and

5. A method of improving the transport of a drug or other active across lipid membr. in the body, or of securing an action as set out in claim 4, characterised by administration of the active in the form of a compound as above.

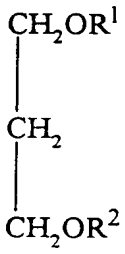
6. A method of manufacture of a medicament for improved therapy involving transport of a drug or other active across lipid membranes in the body, or involving securing an action as set out in claim 4, characterised by the use in, or as, the medicament of a compound as above.

7. A compound or any group of compounds specifically as set out herein, being as claimed in claim 1 or 2.

8. Per se, any compound or group of compounds specifically as set out herein, being novel.

Without limitation of the generality of the foregoing we claim as aspects of the invention:-

1. Compounds of the following structure, when for use in therapy:-



where R^1 is an acyl group derived from a C_{16-30} fatty acid desirably with two or more cis or trans double bonds and particularly an n-6 or n-3 series EFA or conjugated linoleic acid, or columbinic acid, or parinaric acid and R^2 is as R^1 the same or different, or any nutrient, drug or other bioactive residue released as the active in the body.

2. A compound according to claim 1 wherein the fatty acid is gammalinolenic acid, dihomogammalinolenic acid, arachidonic acid, adrenic acid, stearidonic acid, eicosapentaenoic acid, docosapentaenoic acid n-3, or docosahexaenoic acid.

3. A compound according to claim 1 or 2, wherein R^2 is a drug or other active required to cross lipid membranes in the body to exert its action whether in entry to or movement within a cell in which it is to act or in passing the skin, blood-brain or other barrier.

4. A compound according to claim 1 or 2 wherein irrespective of any crossing of lipid membranes R^2 is a drug, vitamin, amino acid, antioxidant or other active which is required to have an action additive to, complementary to, or synergistic with R_1.

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